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Rapid Genomic Fingerprinting of *Lactococcus lactis* Strains by Arbitrarily Primed Polymerase Chain Reaction with ^{32}P and Fluorescent Labels

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Arbitrarily primed polymerase chain reaction, with incorporation of either radioactive or fluorescent labels, was used as a rapid and sensitive method for obtaining genomic fingerprints of strains of *Lactococcus lactis*. Closely related strains produced almost identical fingerprints. Fingerprints of other strains showed only some similarities.

Gram-positive lactic acid bacteria of the genus *Lactococcus* are industrially important. The many different strains that are currently used in the production of cheese are poorly characterized, and the genetic relationships between them are generally not known. The analysis of genomic properties of lactococci promises reliable identification of industrial strains and their derivatives or transconjugants constructed in the laboratory (3, 5, 8). Electrophoretic analysis of the plasmid complements of strains has been described as an identification procedure (3), but the instability of many lactococcal plasmids limits its usefulness. Pulsed-field gel electrophoresis of chromosomal fragments generated by restriction enzyme digestion is a powerful means of strain identification, although the procedures involved take up to a week to complete. Oligonucleotide probes targeted to rRNA sequences can be used to distinguish *Lactococcus lactis* from other bacteria (1), but the highly conserved nature of rRNA sequences does not allow differentiation at the strain level (7).

Arbitrarily primed polymerase chain reaction (AP-PCR) is a novel procedure for genetic analysis which is based on amplification of genomic DNA sequences under low-stringency conditions by using a single oligonucleotide primer. The primer is not targeted toward any specific DNA sequence in the genome. AP-PCR produces a pattern of amplification products that can be used as a genetic fingerprint (11, 13). AP-PCR with ^{32}P label has been used to differentiate between strains of five species of the genus *Staphylococcus* and strains of *Streptococcus pyogenes* (11) and to compare eukaryotic organisms, including *Oryza sativa* (11), *Mus musculus* (12), and *Neurospora crassa* and *Homo sapiens* (13). In this communication, we describe the use of AP-PCR as an effective and rapid method to fingerprint *L. lactis* strains that were previously characterized in our laboratory by pulsed-field gel electrophoresis analysis of *Sma*I restriction digests of genomic DNA (8). We have developed a novel AP-PCR procedure using a fluorescence-labeled primer and an automated DNA sequencer.

L. lactis strains (Table 1) were grown in M17G (M17 medium [9] without lactose but supplemented with 0.5% [wt/vol] glucose) at 30°C. *Escherichia coli* JM107 (Table 1) was grown in 2YT medium (6) at 37°C. Template DNA for AP-PCR was isolated from 0.5 ml of overnight culture. Cells were collected by centrifugation in a 1.5-ml Microfuge tube, resuspended in 0.5 ml of lysis buffer (250 mM NaCl, 10 mM sodium EDTA, 10 mM Tris chloride [pH 8], 10^5 U of lysozyme per ml) and incubated at 37°C for 30 min. Sodium dodecyl sulfate (30 μl of a 10% [wt/vol] solution) was added, and the tube was incubated at 80°C for 5 min. The lysate was mixed with 0.7 ml of phenol-chloroform (1:10) by gentle inversion and centrifuged (5 min at 13,000 × *g*). Nucleic acids were precipitated from the aqueous phase with propan-2-ol (0.7 ml) and pelleted by centrifugation (5 min at 13,000 × *g*). The pellet was dissolved in 50 μl of 10 mM Tris chloride–0.1 mM sodium EDTA (pH 8). This procedure has been used to isolate DNA from *E. coli*, *L. lactis*, and lactic acid bacteria of other genera (*Lactobacillus*, *Leuconostoc*, and *Pediococcus*). DNA concentrations were estimated after electrophoresis through agarose (0.6% [wt/vol]) and ethidium bromide staining by comparison of UV fluorescence with known standards.

AP-PCR with incorporation of ^{32}P was performed by a modification of the method of Welsh and McClelland (11). The initial 10-μl reaction mixtures consisted of 10 ng of template DNA, 5 μM universal M13 primer (5′GTAAAAC GACGGCCAGT3′), 0.03 U of *Taq* polymerase (Promega, Madison, Wis.), and 1× *Taq* polymerase buffer (Promega) adjusted to 4 mM $MgCl_2$ and 0.2 mM (each) deoxynucleoside triphosphate (dNTP). The reaction mixtures were overlaid with 30 μl of mineral oil and subjected to two cycles (Intelligent Heating Block; Hybaid, Teddington, United Kingdom) of 94°C for 5 min, 40°C for 5 min, and 72°C for 5 min. This was followed by 10 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min. The reaction mixture was then replenished by addition of a 30-μl solution containing 1 U of *Taq* polymerase in 1× *Taq* polymerase buffer, 0.2 mM each dNTP, and 0.5 μCi of [α-^{32}P]dATP (Bresatec, Adelaide, Australia); replenishment was followed by 25 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min. The amplification products (5 μl) were resolved through a 6% (wt/vol) polyacrylamide–8 M urea–0.5× TBE (50 mM Tris chloride, 50 mM boric acid, 1 mM sodium EDTA) gel and visualized by autoradiography with an intensifying screen (8 h of exposure at −70°C). AP-PCR with fluorescence-labeled universal M13 primer (2′,7′-dimethoxy-4′,5′-dichloro-6-car-

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TABLE 1. Bacterial strains used in this study

Species and strain	Description	Source and/or reference
L. lactis subsp. *lactis*		
DL11	Prt⁻ derivative of ATCC 11454	D. J. LeBlanc (10)
NCDO712	Commercial starter strain	M. J. Gasson (3)
C2	Commercial starter strain	DRL[a]
LM0230	Plasmid-cured C2	L. L. McKay (4)
L. lactis subsp. *cremoris*		
FG2	Commercial starter strain	DRL
E8	Commercial starter strain	DRL
346	Commercial starter strain	NZDRI[b] (2)
E. coli JM107		New England Biolabs (14)

[a] DRL, Dairy Research Laboratory Collection, Commonwealth Scientific and Industrial Research Organization, Highett, Victoria, Australia.
[b] NZDRI, New Zealand Dairy Research Institute.

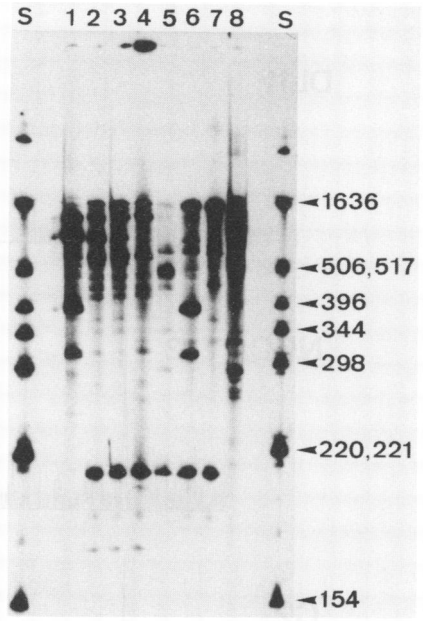

FIG. 1. AP-PCR genomic fingerprints for *L. lactis* DL11 (lane 1), NCDO712 (lane 2), C2 (lane 3), LM0230 (lane 4), FG2 (lane 5), E8 (lane 6), and 346 (lane 7) and *E. coli* JM107 (lane 8). The sizes (in base pairs) of [α-^{32}P]dATP-labeled pBR322 *Hin*fI fragments (S) are shown.

boxy-fluorescein dye primer; Applied Biosystems, Foster City, Calif.) was performed by essentially the same procedure, except that cycles were carried out with a thermal sequencer (model FTS-1C; Corbett Research, Sydney, Australia), 0.2 μM primer was used in the initial 10-μl reaction mixture, the 30-μl replenishment contained 0.075 μM primer instead of [α-^{32}P]dATP, and replenishment was followed by 35 cycles. The fluorescent products were electrophoretically separated and detected with an automated DNA sequencer (ABI model 373A; Applied Biosystems).

Figure 1 shows the genomic fingerprints obtained from AP-PCR with DNA from *L. lactis* strains and *E. coli* JM107. The *L. lactis* strains included three closely related strains, NCDO712 (lane 2), C2 (lane 3), and LM0230 (lane 4). These strains had almost identical AP-PCR fingerprints. Pulsed-field gel electrophoresis analysis (8) showed that these strains also had almost identical *Sma*I restriction patterns.

The AP-PCR fingerprints of strains DL11, FG2, and E8 were recognizably different, although some similarities were apparent (Fig. 1, lanes 1, 5, and 6, respectively). The *Sma*I restriction patterns of FG2 and E8 genomic DNA have also been shown to be clearly different, with some similarities (8). The *Sma*I restriction pattern of DL11 genomic DNA (8) showed no similarity to the FG2 or E8 pattern. The AP-PCR fingerprint of strain 346 (not previously studied by pulsed-field gel electrophoresis) also showed a mixture of similarities to and differences from the other fingerprints (lane 7).

One distinctive feature of some of the *L. lactis* fingerprints was a common ca. 200-bp AP-PCR product. This AP-PCR product was absent from the fingerprint of strain DL11 (Fig. 1, lane 1) and also from the fingerprints of *L. lactis* subsp. *lactis* C6 and C10 (data not shown).

These data illustrated the ability of AP-PCR to detect strain relationships and to differentiate unambiguously between strains that are not closely related. The AP-PCR fingerprint of *E. coli* JM107 (lane 8) showed no similarity to those of any of the lactococcal strains. AP-PCR was also performed with different amounts of template (0.1, 0.5, 1, and 5 ng), and the results (data not shown) were qualitatively the same as those shown in Fig. 1. Some variations in band intensities and the numbers and sizes of AP-PCR products were observed, especially at the lower template concentrations. Similar variations have been reported for genomic fingerprints of staphylococci (11).

Figure 2 shows a comparison of genomic fingerprints of DNA from four *L. lactis* strains, obtained via electrophoresis and detection of fluorescent AP-PCR products with an automated DNA sequencer. The pattern of fluorescence peaks corresponded to the pattern of bands in Fig. 1. Strains NCDO712 and C2 gave very similar patterns, which is consistent with the close relationship between these strains (3). The fingerprint of strain LM0230 (a mutagenized, plasmid-cured derivative of C2) (4) was similar but showed some differences (Fig. 2). All three fingerprints contained a peak corresponding to a product of an estimated 210 bp, presumably corresponding to the ca. 200-bp product observed with ^{32}P label. This product was absent from the DL11 fingerprint. Thus the results obtained with a fluorescent primer were comparable with the results with ^{32}P label, but it is clear from Fig. 1 and 2 that a larger number of AP-PCR products can be resolved by using the fluorescence detection system, suggesting a potential for more sensitive discrimination between related strains. The automated DNA sequencer allows computer storage of data, providing the basis for the compilation of a reference library of fingerprints.

Pulsed-field gel electrophoresis of genomic DNA restriction fragments can be used in strain identification (5, 8), and it also gives information that can be used in further genetic characterization (10). However, the simplicity and speed of AP-PCR (results are obtained in less than 2 days) suggest that it can provide a convenient and effective method for genetic fingerprinting of lactococci. This approach should be readily applicable to other lactic acid bacteria.

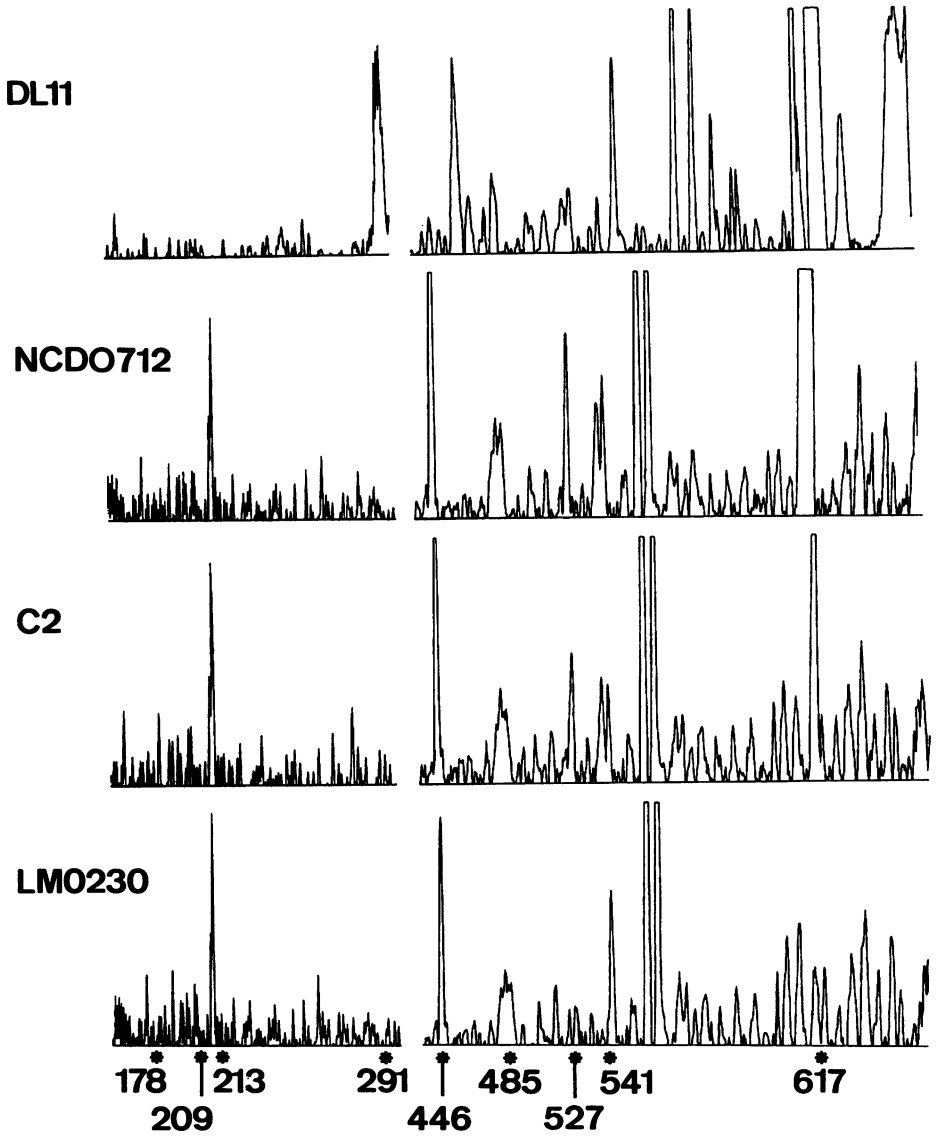

FIG. 2. AP-PCR genomic fingerprints for *L. lactis* DL11 (compare with Fig. 1, lane 1), NCDO712 (Fig. 1, lane 2), C2 (Fig. 1, lane 3), and LM0230 (Fig. 1, lane 4). Selected regions of the fluorescence output tracing from the DNA sequencer are shown. Size markers (in base pairs) were derived from internal standards of M13mp18 DNA sequenced with fluorescence-labeled universal M13 primer (6-carboxy-X-rhodamine dye primer; Applied Biosystems).

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